BIGLYCAN KNOCKOUT MICE SHOW ALTERED BIOMINERALIZATION

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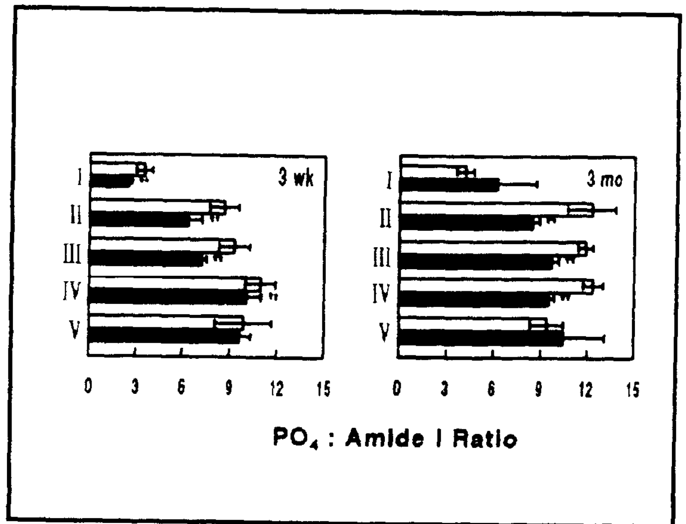
Clinical Relevance
This study, based on a genetically engineered mutant mouse, presents evidence that biglycan is important for initiation of calcification, providing a biochemical explanation for the short stature seen in Turner's syndrome, in which biglycan expression is depressed, and for the excessive growth (i.e., tall stature) seen in Kleinfelder's syndrome in which biglycan expression is elevated.

Introduction
Biglycan (BGN) is a small leucine-rich proteoglycan consisting of a ~40,000 MW core protein and two GAG chains: chondroitin sulfate in bone, skin, and tendon, or dermatan sulfate in cartilage. Similar to the other small proteoglycan found in these tissues, decorin, BGN can bind growth factors and regulate collagen fibrillogenesis *in vitro*. Its *in situ* functions are unknown. Based on the under expression of the BGN gene in Turner's syndrome and its over expression in Kleinfelder's syndrome [1], along with the ability of BGN to increase apatite formation *in vitro* [2], BGN has been proposed to play a role in the regulation of initial mineralization. Mice lacking the BGN core protein gene provided the opportunity to test this hypothesis.

Materials and Methods
Mice lacking the BGN gene (k.o. mice) were generated using ES technology by insertion of a neomycin resistance gene into exon 2, and heterozygous mutants were bred to provide knockout (k.o.) and wild type (w.t.) animals for this study. Western analysis confirmed that the k.o. mice did not make BGN, and, further showed an increased accumulation of decorin protein in skin, cartilage, sternum and heart tissues of the k.o. mice. Genotyping of mice used in study was based on Southern blotting. Animals were sacrificed at 3 wks (n=4 per genotype) and 3 mos (n=3 per genotype). Tibia, fixed in ethanol were subjected to fine-focus radiography, and were then embedded in Spurr's resin. Five 0.5μ thick sections per bone were cut and mounted on BaF_2 windows for Fourier transform infrared (FT-IR) microscopy. Additional sections stained with toluidine blue were used for orientation. Spectra were recorded using a 20x20μ aperture going across the cortical bone from the periosteum to endosteum, across the cancellous bone, and the calcified and non-calcified cartilage regions of the epiphyseal plate. For each bone a minimum of 40 spectra were recorded. These were analyzed to provide integrated areas of the v_1v_3 phosphate mode (900-1200 cm^{-1}), the amide I vibrations (1585-1715 cm^{-1}), and the v_2 carbonate mode (860-885 cm^{-1}). Ratios of phosphate:amide I, a parameter proportional to ash weight [3], and carbonate:phosphate ratio, which reflects mineral crystal maturity were calculated. The complex carbonate and phosphate bands were curve-fit to provide insights into the types of carbonate substitutions present, and the crystallinity of the mineral [4].

Results and Discussion
From the radiographs and histology, no significant changes were noted, however the k.o. bones at both 3 wks and 3 mos appeared disorganized. At both ages, the phosphate to amide I ratio (figure) of the w.t. bones was generally greater than that of the same site in the k.o. bones. Significant differences were seen in the calcified cartilage, cancellous bone, and periosteum, all sites of new mineral deposition, at both ages. Further, the crystallinity in the k.o. bones (measured in terms of four different curve-fit parameters) was greater than the w.t. bones at the same site and age. The parameters used to assess crystallinity were percent areas of the 1075 cm^{-1} subband, the 1060 cm^{-1} subband, the 1045 cm^{-1} subband, and the ratio of the areas of the 1020 to 1030 cm^{-1} subbands [4]. Carbonate to phosphate ratios were not significantly different, however the % labile carbonate was lower in the newly mineralized regions of the k.o. bones as contrasted with these regions in the w.t. bones. For example, at 3 mo, while the carbonate at all sites examined was substituted predominately for phosphate (64±5% (sem) in both w.t. and k.o.), the periosteum in the w.t. had 32±3% labile carbonate, while in the k.o. there was 15±1.8% labile carbonate.



Mineral content measured by FTIR at discrete sites in the tibia. Mean ± SEM for knockout (solid bar) and wild type (open bars) in the calcified cartilage (I), cancellous bone (II), periosteum (III), central cortex (IV), and endosteum (V). ** $p < 0.05$ w.t. vs. k.o. at same anatomic site.

These results are compatible with a mechanism in which BGN is one of several matrix proteins that promote initial mineralization. When BGN is absent, there appear to be fewer sites for new mineral deposition. These fewer sites may result from the smaller collagen fibril size noted in the k.o., as well as from the absence of the protein which acts *in vitro* as an apatite nucleator [2]. In the presence of fewer new nucleation sites, the crystals which are present grow to a larger size than normal, but the total mineral content is reduced, and the matrix organization disrupted. The decreased collagen fibril size and the retarded initial mineralization can explain the short stature and premature osteoporosis present in humans with reduced BGN expression, and the tall stature seen when the gene is overexpressed. The absence of a distinctive phenotype in the mouse at early ages is evidence of the redundant nature of the proteins involved in mineralization.

References
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Acknowledgments: Supported by NIH DE04141. The authors would like to thank Dr. S.B. Doty and Ms. K. Bock for their assistance with the histology and section preparation.

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